

**The University of Kansas (KU)
Animal Care and Use Program**

**Standard Operating Procedure
CO₂ Euthanasia of Rodents**

1.0 Scope and Application

This procedure applies to all animal care personnel, technical staff, and investigators and provides basic instruction for the use of carbon dioxide for euthanasia of rodents.

2.0 Summary of Method

- **Euthanasia of Fetuses**
 - When fetuses are not required for study, euthanasia of the dam should ensure rapid death of the fetuses. Follow the procedures for Euthanasia of Adult Rodents.

- **Euthanasia of Adult and Neonatal Rodents**
 - When possible, the home cage should be used to transport animals to the euthanasia station.
 - Ensure flow meter is in the off position before opening the CO₂ valve.
 - Open the valve on the CO₂ cylinder.
 - The cage should be filled at 20% of its volume per minute. Calculate the volume of the cage in liters and multiply this value by 0.2 to determine the flow rate needed:

$$\text{Volume} = \frac{\text{length (cm)} \times \text{height (cm)} \times \text{width (cm)}}{1000} = \text{Volume in liters}$$

$$\text{Volume in liters} \times 0.2 = \text{flow rate of CO}_2 \text{ in Liters Per Minute (LPM)}$$

- **If using the cages supplied by the Animal Care Unit the flow rates are as follows:**

Cage Type	Flow (LPM)
Mouse Standard Cage	1.5
Mouse Large Cage	4
Rat Standard Cage	5

- Remove microisolator top (if applicable) and place euthanasia lid over cage. Turn on the gas and adjust the flow meter to the calculated flow rate to release CO₂.
- Allow the animals at least 2-3 minutes of exposure before removing from the euthanasia chamber.
- Neonatal mice and rats (up to 10 days) are resistant to hypoxia. The duration of exposure to carbon dioxide varies and may require exposures as long as 1 hour to ensure euthanasia.
- Carefully check each animal for heartbeat, respiration or any other signs of life. If the animal is still alive recharge the tank with CO₂. Recheck each animal to ensure euthanasia.

- For pups less than 10 days of age perform either cervical dislocation or decapitation with sharp scissors on each animal after removal from the euthanasia chamber.
- For all other rodents, cervically dislocate (animals under 200 g only) or perform a thoracotomy on each animal after removal from the euthanasia chamber.
- Place all animals into a plastic bag labeled with species, animal ID, date, and the investigator's name. If a necropsy is requested, place the body in the refrigerator. Animal carcasses should be placed in the freezer for disposal purposes.
- Close the valve on the CO₂ cylinder completely.
- Reduce pressure on flow meter and exhaust excess gas by turning the flow meter counterclockwise until the balls falls to zero and then turn off by turning in the opposite direction.
- Commercial euthanasia systems (i.e. Euthanx) must be used according to manufacturer directions. Clear concise directions for proper use are posted or readily available near these systems.

References

1. American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2013 Edition. Retrieved from <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. Artwohl J, Brown P, Corning B, Stein S. (August 2005). Report of the ACLAM Task Force on Rodent Euthanasia. Retrieved from http://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf.
3. Neil L, Weary DM. Behavioral responses of the rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. Applied Animal Behavior Science 100 (2006) 295-308.
4. National Institute of Health. Animal Research Advisory Council. "Guidelines for Euthanasia of Rodents Using Carbon Dioxide". https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_euthanasia_adult.pdf

Revision History

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